Food and Chemical Toxicology 49 (2011) 305–308

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Inhibition of hepatic δ-aminolevulinate dehydratase activity induced by mercuric chloride is potentiated by *N*-acetylcysteine *in vitro*

Ricardo Brandão *, Cristina W. Nogueira

Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

ARTICLE INFO

Article history:
Received 12 March 2010
Accepted 29 October 2010

Keywords:
Mercury
N-acetylcysteine
δ-Aminolevulinate dehydratase
Lipid peroxidation

ABSTRACT

Mercuric chloride $(HgCl)_2$ is a toxic metal that causes oxidative damage in several tissues. *N*-acetylcysteine (NAC) is a sulfhydryl compound with antioxidant activity. In the present study, we investigated the *in vitro* effects of the association between $HgCl_2$ and NAC in tissues of mice. For this purpose, we evaluated the *in vitro* effect of $HgCl_2$ + NAC association on δ-aminolevulinate dehydratase (δ-ALA-D) activity and on thiobarbituric acid reactive substances (TBARS) levels in liver and kidney of mice. The results demonstrate that $HgCl_2$ inhibited δ-ALA-D activity in both tissues. Hepatic δ-ALA-D activity inhibited by $HgCl_2$ was potentiated by the highest concentration of NAC. The inhibition of hepatic δ-ALA-D activity seems to be related to sulfhydryl groups oxidation of the enzyme. We observed also that $HgCl_2$ increased TBARS levels in kidney and liver. Hepatic TBARS levels were reduced by NAC, at higher concentration. In contrast, NAC, at higher concentration, increased renal TBARS levels. In conclusion, the inhibition of hepatic δ-aminolevulinate dehydratase activity induced by $HgCl_2$ is potentiated by NAC *in vitro*, and this effect is not related to hepatic lipid peroxidation.

1. Introduction

Inorganic mercury is widely used in certain types of batteries and continues to be an essential component of fluorescent light bulbs (Clarkson, 1997). It is known that mercury promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxide. ROS enhance the subsequent iron- and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical (Hussain et al., 1999). These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell. Inorganic mercury also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase and glutathione peroxidase (Benov et al., 1990).

δ-Aminolevulinate dehydratase (δ-ALA-D; EC 4.2.1.24) is very sensitive to agents which oxidizing sulfhydryl groups, including mercury (Emanuelli et al., 1996). This enzyme plays a fundamental role in most aerobic organisms by participating in heme biosynthesis pathway (Sassa et al., 1989). The enzyme inhibition can lead to δ-ALA (δ-aminolevulinic acid) accumulation, which in turn can enhance the generation of free radicals, aggravating oxidative damage to cellular components (Pereira et al., 1992; Bechara, 1996).

N-acetylcysteine (NAC) is used clinically as a mucolytic agent administered by inhalation and also is recognized as effective for the treatment of acetaminophen intoxication (Ziment, 1986; Borgström et al., 1986). It has been shown that NAC is effective as a chelating agent in reducing the toxicity of heavy metals (Banner et al., 1986; Ottenwalder and Simon, 1987; Girardi and Elias, 1991). Moreover, NAC, a power antioxidant, is a glutathione (GSH) precursor and directly eliminates ROS (Aruoma et al., 1989). In fact, Pivetta et al. (2006) has demonstrated that NAC is effective in protecting against oxidative damage induced by ethanol in mice liver. However, Sprong et al. (1998) have reported that a high-dose of NAC (950 mg/kg, in 48 h) increased lipopolysaccharide-induced lung injury. Oxidative damage has been also demonstrated after NAC administration (Okiawa et al., 1999).

Previous study of our research group showed that NAC potentiates the toxicity induced by mercuric chloride ($HgCl_2$) in mice (Brandão et al., 2006). Based on the fact that ($HgCl_2$) causes oxidative damage, the present study was designed to determine the effect of NAC on the toxicity caused by $HgCl_2$ in mice. To this end, we evaluated the *in vitro* effect of $HgCl_2$ + NAC on δ-ALA-D activity and on Thiobarbituric acid reactive substances (TBARS) levels in liver and kidneys of mice.

2. Materials and methods

2.1. Chemicals

($HgCl_2$) was obtained from Merck (Darmstadt, Germany). NAC, dithiothreitol (DTT), δ-ALA and *p*-dimethylaminobenzaldehyde were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

* Corresponding author. Tel.: +55 55 3220 8140; fax: +55 55 3220 8978.
E-mail address: ricardo_br79@yahoo.com.br (R. Brandão).

0278-6915 Published by Elsevier Ltd. Open access under the Elsevier OA license.
doi:10.1016/j.fct.2010.10.033

2.2. Animals

Male adult Swiss albino mice (2 months old, 25–35 g) from our own breeding colony were used. The animals were kept on a separate animal room 1 week before experimental procedure, on a 12 h light/dark cycle, at temperature of 22 ± 2 °C, with free access to food and water. This study was approved by the Ethics and Animal Welfare Committee of Universidade Federal de Santa Maria.

2.3. Tissue preparation

Mice were anesthetized and killed by decapitation. Samples of kidney and liver were rapidly homogenized in 50 mM Tris–Cl, pH 7.4 (1/10, w/v) and kept on ice. The homogenate was centrifuged at 2400× *g* for 15 min to yield a low-speed supernatant (S1) fraction. S1 freshly prepared was used for δ-ALA-D and TBARS assays. Two mice were used by assay.

2.4. δ-ALA-D activity

δ-ALA-D activity was assayed by the method of Sassa (1982) by measuring the rate of product (porphobilinogen, PBG) formation except that 100 mM potassium phosphate buffer, pH 6.8 and 2.4 mM of δ-ALA were used (Barbosa et al., 1998). After a pre-incubation period, enzymatic reaction was initiated by adding the substrate (δ-ALA) in the medium and incubated for 1 h at 37 °C. The incubation was stopped by adding trichloroacetic acid solution (10% TCA) with 10 mM $HgCl_2$. PBG, which is formed within a fixed time, is mixed with modified Ehrlich's reagent, and the color developed is measured photometrically (555 nm) against a blank. The reaction product was determined using modified Ehrlich's reagent at 555 nm, with a molar absorption coefficient of 6.1×10^4 per M for the Ehrlich-porphobilinogen salt. Results were reported as nmol PBG/mg protein/h.

2.4.1. Pre-incubation period

S1 from liver and kidneys was pre-incubated for 10 min at 37 °C in the presence of different concentrations of $HgCl_2$ (25 or 50 μM to liver and 10 or 25 μM to kidney) and NAC (10–500 μM). After, the reaction was initiated as described above. These concentrations were chosen based on previous studies of our research group (data not shown).

2.4.2. Effect of dithiothreitol (DTT) on δ-ALA-D activity

To assess the capacity of DTT to restore δ-ALA-D inhibition induced by $HgCl_2$ + NAC, an aliquot of S1 was pre-incubated with different concentrations of $HgCl_2$ (50 μM) and NAC (500 μM) for 10 min at 37 °C. After that, the reaction was started by the addition of substrate (δ-ALA) followed immediately by the addition of 3 mM DTT. This assay was performed according to the technique described above (item 2.4).

2.5. Determination of TBARS

An aliquot (200 μL) of S1 of liver and kidney was pre-incubated at 37 °C for 1 h with different concentrations of $HgCl_2$ (10–200 μM) and NAC (50 or 500 μM). These concentrations were chosen based on previous studies of our research group (data not shown). The reaction product was determined using 500 μL thiobarbituric acid (0.8%), 200 μL sodium dodecil sulphate (SDS) 8.1% and 500 μL acetic acid (pH 3.4) and incubated at 95 °C for 2 h. TBARS were determined as described by Ohkawa et al. (1979). Results were reported as nmol MDA (malondialdehyde)/mg protein.

2.6. Protein quantification

Protein was measured by the method of Bradford (1976) using bovine serum albumin as standard.

2.7. Statistical analysis

Data are expressed as mean ± S.D. All results were analyzed by two-way ANOVA, followed by Duncan's Multiple Range Test when appropriate. Main effects are presented only when the higher second order interaction was non-significant. Differences were considered to be significant when $p < 0.05$.

3. Results

3.1. δ-ALA-D activity

Two-way ANOVA of hepatic δ-ALA-D activity showed a significant interaction between $HgCl_2$ and NAC ($F_{10,12} = 2.85$, $p < 0.003$). The association of $HgCl_2$ and NAC, at non-inhibitory concentrations (25 and 500 M, respectively), inhibited hepatic δ-ALA-D activity. $HgCl_2$, at the concentration of 50 M, inhibited hepatic δ-ALA-D activity (~32%) when compared to control tube. δ-ALA-D activity inhibition induced by 50 M $HgCl_2$ was increased by NAC at the concentration of 500 M (Fig. 1).

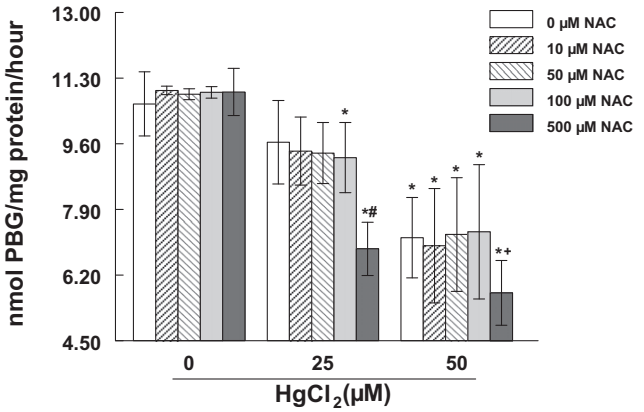

Fig. 1. Effect of $HgCl_2$ and NAC on δ-ALA-D activity from mouse liver. Results are expressed as PBG/mg protein/h. Data represent the mean ± S.D. of 4 to 6 experiments. *Denotes $p < 0.05$ as compared to control (without NAC or $HgCl_2$) (two-way ANOVA/Duncan). #Denotes $p < 0.05$ as compared to 25 μM $HgCl_2$ (two-way ANOVA/Duncan). +Denotes $p < 0.05$ as compared to 50 μM $HgCl_2$ (two-way ANOVA/Duncan).

DTT, a sulfhydryl reducing agent, completely recovered δ-ALA-D activity inhibited by $HgCl_2$ + NAC in homogenate of mouse liver (Table 1).

Two-way ANOVA of renal δ-ALA-D activity did not reveal interaction between $HgCl_2$ and NAC ($F_{8,76} = 0.28$, $p < 0.969$). However, $HgCl_2$ presented a main effect ($p < 0.001$) on renal δ-ALA-D activity. Renal δ-ALA-D activity was inhibited by $HgCl_2$ at concentrations of 10 and 25 μM (~35 and 60%, respectively) when compared to the control tube. The inhibition induced by $HgCl_2$ in renal δ-ALA-D activity was not modified by NAC addition (Fig. 2).

3.2. TBARS

Two-way ANOVA of hepatic TBARS levels did not reveal interaction between $HgCl_2$ and NAC ($F_{6,86} = 1.74$, $p < 0.122$). However, $HgCl_2$ and NAC presented a main effect ($p < 0.001$) on hepatic TBARS levels. $HgCl_2$ at concentrations of 50 and 200 μM induced an increase in hepatic TBARS levels (~49 and 77%, respectively). NAC, at the concentration of 500 μM, protected about 30% against the increase in hepatic TBARS levels induced by 50 μM $HgCl_2$. In addition, NAC at the concentration of 500 μM reduced per se TBARS levels (~20%) (Fig. 3).

In renal tissue, NAC at concentration of 500 μM per se caused a pro-oxidant effect (~31%), when compared to the control tube. $HgCl_2$ at the concentration of 200 μM induced an increase in TBARS levels (~35%) when compared to the control tube (Fig. 4).

4. Discussion

The results of the present study demonstrate that the association between NAC and $HgCl_2$, at non-inhibitory concentrations, caused an inhibition in hepatic δ-ALA-D activity. In addition, the inhibition caused by $HgCl_2$ was potentiated by the highest concentration of NAC. These results clearly demonstrate a harmful effect of the association between $HgCl_2$ and NAC on the hepatic δ-ALA-D activity. It is possible that Hg^{2+} causes Zn^{2+} displacement, leading to δ-ALA-D inhibition. In fact, mammalian δ-ALA-D is a metalloenzyme that requires Zn^{2+} for maximal catalytic activity and data support the hypothesis of direct competition between bivalent metals and Zn^{2+} in δ-ALA-D activity (Nogueira et al., 2004). In

Table 1
δ-ALA-D activity in liver, with or without dithiothreitol (DTT).

	Hepatic δ-ALA-D (without DTT)	Hepatic δ-ALA-D (with DTT)
Control	11.05 ± 0.17	11.05 ± 0.17
$HgCl_2$ 50 μM	7.40 ± 1.13[a]	11.76 ± 0.47
NAC 500 μM	10.94 ± 0.61	10.94 ± 0.61
$HgCl_2$ 50 μM + NAC 500 μM	5.79 ± 0.96[a,b]	11.58 ± 0.31

Data are mean ± S.D. from 4 to 6 experiments.
[a] Denoted $p < 0.05$ as compared to the control group (two-way ANOVA/Duncan).
[b] Denoted $p < 0.05$ as compared to the $HgCl_2$ 50 M and NAC 500 M groups (two-way ANOVA/Duncan).

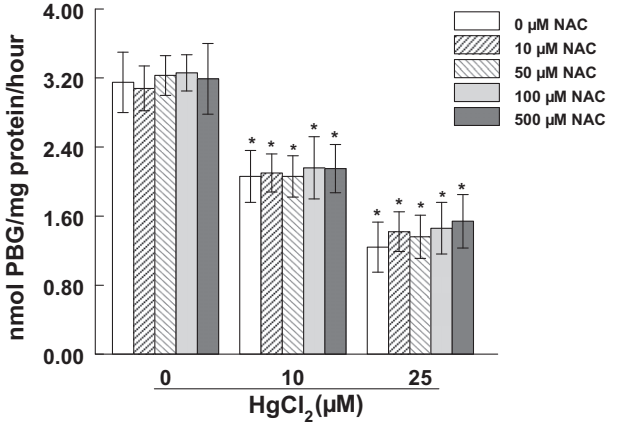

Fig. 2. Effect of $HgCl_2$ and NAC on δ-ALA-D activity from mouse kidney. Results are expressed as nmol PBG/mg protein/h. Data represent the mean ± S.D. of 4 to 6 experiments. *Denotes $p < 0.05$ as compared to control (without NAC or $HgCl_2$) (two-way ANOVA/Duncan).

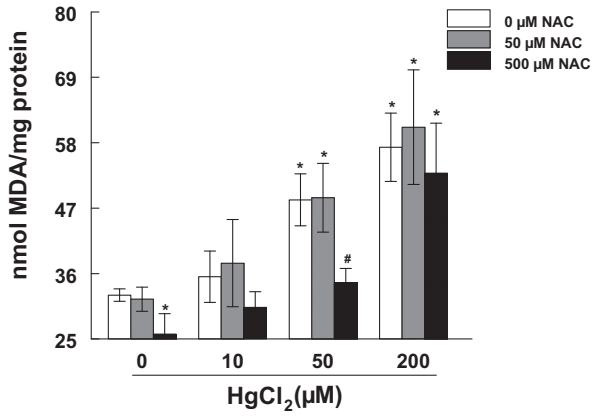

Fig. 3. Effect of $HgCl_2$ and NAC on TBARS levels from mouse liver. Results are expressed as nmol malondialdehyde (MDA)/mg protein. Data represent the mean ± S.D. of 4 to 6 experiments. *Denotes $p < 0.05$ as compared to control (without NAC or $HgCl_2$) (two-way ANOVA/Duncan). #Denotes $p < 0.05$ as compared to 50 μM $HgCl_2$ (two-way ANOVA/Duncan).

addition, it is well known that mercury have a high affinity for sulfhydryl groups of proteins and other endogenous biomolecules (Clarkson, 1997; Zalups and Barfuss, 1998). In fact, several studies have demonstrated that $HgCl_2$ inhibited δ-ALA-D activity in different tissues by oxidation of sulfhydryl groups located at the active site of the enzyme (Rocha et al., 1995; Farina et al., 2003). In according, Dorward and Yagminas (1994) demonstrated that erythrocyte δ-ALA-D activity from female cynomolgus monkeys was increased after exposure *in vitro* to DTT or zinc. In addition, we

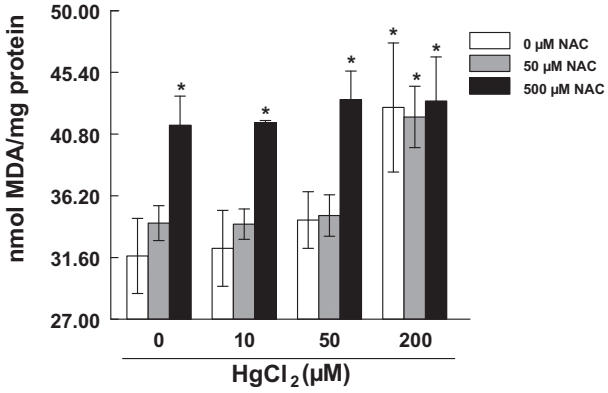

Fig. 4. Effect of $HgCl_2$ and NAC on TBARS levels from mouse kidney. Results are expressed as nmol malondialdehyde (MDA)/mg protein. Data represent the mean ± S.D. of 4 to 6 experiments. *Denoted $p < 0.05$ as compared to control (without NAC or $HgCl_2$) (two-way ANOVA/Duncan).

have reported, in an *ex vivo* study, that combined administration of NAC and $HgCl_2$ inhibited renal Na^+, K^+-ATPase in mice (Brandão et al., 2006). In addition to δ-ALA-D, Na^+, K^+-ATPase is also a sulfhydryl-containing enzyme and, consequently, can be sensitive to oxidizing agents (Carfagna et al., 1996; Folmer et al., 2004). Therefore, the data found in this *in vitro* study reinforce the potential toxicity of $HgCl_2$ and NAC demonstrated *ex vivo*. To the best of our knowledge this is the first time that association of NAC + $HgCl_2$ is reported in the literature as an inhibitor of δ-ALA-D activity.

NAC is a thiol-containing antioxidant (Moldeus et al., 1986) and ROS scavenging (Aruoma et al., 1989). Moreover, Banner et al. (1986) have indicated that NAC has also chelating activity with regard to diverse heavy metals. Evidence demonstrated that the formation of ternary complexes between metals, chelators and enzyme can be only inferred from the inhibition of δ-ALA-D activity (Markham et al., 1993). In this study, the inhibition of hepatic δ-ALA-D activity by NAC + $HgCl_2$ association was avoided by DTT, suggesting that the mechanism responsible for the enzyme inhibition involves the oxidation of sulfhydryl groups. Thus, we can suggest an oxidant potential of a possible complex formed between $HgCl_2$ and NAC on δ-ALA-D activity.

On the renal tissue, δ-ALA-D activity was more sensitive to $HgCl_2$ exposure than in the liver (10 μM was able to inhibit the enzyme activity in renal tissue, whereas 50 μM inhibited the hepatic δ-ALA-D activity). In contrast to liver, in the kidney, the association of $HgCl_2$ + NAC did not cause a higher inhibitory effect on δ-ALA-D activity.

Lipoperoxidation has been reported after *in vitro* and *in vivo* exposure to mercury (Lund et al. 1993; Perottoni et al., 2004). In this regard, we observed that $HgCl_2$ caused an increase in hepatic and renal TBARS levels. The antioxidant action of NAC is believed to originate from its ability to scavenging ROS (Aruoma et al., 1989). Accordingly, we observed that NAC at the highest concentration protected against the increase of hepatic TBARS levels induced by $HgCl_2$ at 50 μM. In contrast, we verified that the increase in renal TBARS levels caused by 200 μM $HgCl_2$ was not modified by NAC.

We verified also that NAC at the highest concentration decreased basal hepatic TBARS levels. In contrast, we observed that NAC at 500 μM increased per se the renal TBARS levels. In fact, some studies have demonstrated that NAC, at the high doses, could induce toxicity by increasing oxidative stress (Held and Biaglow, 1994; Sprong et al., 1998).

The results obtained in this study point out a different action of $HgCl_2$ + NAC association in lipid peroxidation and δ-ALA-D activity dependent on the tissue evaluated and concentration used. NAC

potentiated the hepatic δ-ALA-D activity inhibition induced by $HgCl_2$. δ-ALA-D inhibition could be not related to lipid peroxidation, since NAC reduced lipid peroxidation induced by $HgCl_2$ in liver. In contrast, we did not observe the existence of an interaction between $HgCl_2$ and NAC in renal tissue.

In conclusion, these results indicate that NAC potentiated the inhibition of hepatic δ-ALA-D activity induced by $HgCl_2$ *in vitro* which is not related to lipid peroxidation. The harmful effects of $HgCl_2$ + NAC were dependent on the concentration and the tissue analyzed.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The financial support by FAPERGS, CAPES and CNPQ is gratefully acknowledged.

References

Aruoma, O.I., Halliwell, B., Hoey, B.M., Butler, J., 1989. The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. Free Radic. Biol. Med. 6, 593–597.

Banner, W.J.R., Koch, M., Capin, D.M., Hopf, S.B., Chang, S., Tong, T.G., 1986. Experimental chelation therapy in chromium, lead and boron intoxication with *N*-acetylcysteine and other compounds. Toxicol. Appl. Pharmacol. 83, 142–147.

Barbosa, N.B.V., Rocha, J.B.T., Zeni, G., Emanuelli, T., Beque, M.C., Braga, A.L., 1998. Effect of organic forms of selenium on δ-aminolevulinate dehydratase from liver, kidney and brain of adults rats. Toxicol. Appl. Pharmacol. 149, 243–253.

Bechara, E.J., 1996. Oxidative stress in acute intermittent porphiria and lead poisoning may be triggered by 5- aminolevulinic acid. Braz. J. Med. Biol. Res. 29, 841–851.

Benov, L.C., Benchev, I.C., Monovich, O.H., 1990. Thiol antidotes effect on lipid peroxidation in mercury-poisoned rats. Chem. Biol. Interact. 76, 321.

Borgström, L., Kagedal, B., Paulsen, O., 1986. Pharmacokinects of *N*-acetylcysteine in man. Eur. J. Clin. Pharmacol. 31, 217–222.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brandão, R., Santos, F.W., Zeni, G., Rocha, J.B.T., Nogueira, C.W., 2006. DMPS and *N*-acetylcysteine induced renal toxicity in mice exposed to mercury. Biometals 19, 389–398.

Carfagna, M.A., Ponsler, G.D., Muhoberac, B.B., 1996. Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals. Chem. Biol. Interact. 100, 53–65.

Clarkson, T.W., 1997. The toxicology of mercury. Crit. Rev. Clin. Lab. Sci. 34, 369–403.

Dorward, A., Yagminas, A.P., 1994. Activity of erythrocyte δ-aminolevulinic acid dehydratase in the female cynomolgus monkey (*Macaca fascicularis*): kinetic analysis in control and lead-exposed animals. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 108, 241–252.

Emanuelli, T., Rocha, J.B.T., Pereira, M.E., Porciúncula, L.O., Morsch, V.M., Martins, A.F., Souza, D.O.G., 1996. Effect of mercury chloride intoxication and dimercaprol treatment on delta-aminolevulinate dehydratase from brain, liver and kidney of adult mice. Pharmacol. Toxicol. 79, 136–143.

Farina, M., Brandão, R., Lara, F.S., Soares, F.A.A., Souza, D.O., Rocha, J.B.T., 2003. Profile of nonprotein thiols, lipid peroxidation and delta-aminolevulinate dehydratase activity in mouse kidney and liver in response to acute exposure to mercuric chloride and sodium selenite. Toxicology 184, 179–187.

Folmer, V., Santos, F.W., Savegnago, L., Brito, V.B., Nogueira, C.W., Rocha, J.B.T., 2004. High sucrose consumption potentiates the sub-acute cadmium effect on Na^+-K^+-ATPase but not on and δ-aminolevulinate dehydratase in mice. Toxicol Lett. 153, 333–341.

Girardi, G., Elias, M.M., 1991. Effectiveness of *N*-acetylcysteine in protecting against mercuric chloride-induced nephrotoxicity. Toxicology 67, 155–164.

Held, K.D., Biaglow, J.E., 1994. Mechanisms for the oxygen radical mediated toxicity of various thiol-containing compounds in cultured mammalian cells. Radiat. Res. 139, 15–23.

Hussain, S., Atkinson, A., Thompson, S.J., Khan, A.T., 1999. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver and kidneys of mice. J. Environ. Sci. Health B 34, 645–660.

Lund, B.O., Miller, M.D., Woods, J.S., 1993. Studies on Hg(II)-induced H_2O_2 formation and oxidative stress *in vivo* and *in vitro* in rat kidney mitochondria. Biochem. Pharmacol. 45, 2017–2024.

Markham, G.D., Myers, C.M., Harris, K.A., Volin, J.R.M., Jaffe, E.K., 1993. Spatial proximity and sequence localization of the reactive sulfhydryls of porphobilinogen synthase. Prot. Sci. 2, 71–79.

Moldeus, P., Cotgreave, I.A., Berggren, M., 1986. Lung protection by a thiol-containing antioxidant: *N*-acetylcysteine. Respiration 50, 31–42.

Nogueira, C.W., Santos, F.W., Soares, F.A., Rocha, J.B.T., 2004. 2, 3-Dimercaptopropane-1-sulfonic acid and meso-2, 3-dimercaptosuccinic acid inhibit δ-aminolevulinate dehydratase from human erythrocytes in vitro. Environ. Res. 94, 254–261.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Okiawa, S., Yamada, K., Yamashita, N., Tada-Oikawa, S., Kawanishi, S., 1999. *N*-acetylcysteine, a cancer chemopreventive agent, causes oxidative damage to cellular and isolated DNA. Carcinogenesis 20, 1485–1490.

Ottenwalder, H., Simon, P., 1987. Differential effect of *N*-acetylcysteine on excretion of the metals Hg, Cd, Pb and Au. Arch. Toxicol. 60, 401–402.

Pereira, B., Curi, R., Kokubun, E., Bechara, E.J., 1992. 5-Aminolevulinic acid-induced alterations of oxidative metabolism in sedentary and exercise-trained rats. J. Appl. Physiol. 72, 226–230.

Perottoni, J., Rodrigues, O.E.D., Paixão, M.W., Zeni, G., Lobato, L.P., Braga, A.L., Rocha, J.B.T., Emanuelli, T., 2004. Renal and hepatic ALA-D activity and selected oxidative stress parameters of rats exposed to inorganic mercury and organoselenium compounds. Food Chem. Toxicol. 42, 17–28.

Pivetta, L.A., Pereira, R.P., Farinon, M., de Bem, A.F., Perottoni, J., Soares, J.C., Duarte, M.M.F., Zeni, G., Rocha, J.B.T., Farina, M., 2006. Ethanol inhibits δ-aminolevulinate dehydratase and glutathione peroxidase activities in mice liver: protective effects of ebselen and *N*-acetylcysteine. Environ. Toxicol. Pharmacol. 21, 338–343.

Rocha, J.B.T., Pereira, M.E., Emanuelli, T., Christofari, R.S., Souza, D.O., 1995. Effects of mercury chloride and lead acetate treatment during the second stage of rapid postnatal brain growth on ALA-D activity in brain, liver, kidney and blood of suckling rats. Toxicology 100, 27–37.

Sassa, S., 1982. Delta- aminolevulinic acid dehidratase assay. Enzyme 28, 133–145.

Sassa, S., Fujita, H., Kappas, A., 1989. Genetic and chemical influences on heme biosynthesis. In: Kotyk, A., Skoda, J., Paces, V., Kostka, V. (Eds.), Highlights of Modern Biochemistry. VSP, Utrecht, pp. 329–338.

Sprong, R.C., Winkelhuyzen-Janssen, A.M.L., Aarsman, C.J.M., van Oirschot, J.F.L.M., van der Bruggen, T., van Asbeck, B.S., 1998. Low dose *N*-acetylcysteine protects rats against endotoxin-mediated oxidative stress, but high-dose increases mortality. Am. J. Respir. Crit. Care Med. 157, 1283–1293.

Zalups, R.K., Barfuss, D.W., 1998. Participation of mercuric conjugates of cysteine, homocysteine and *N*-acetylcysteine in mechanisms involved in the renal tubular uptake of inorganic mercury. J. Am. Soc. Nephrol. 9, 551–561.

Ziment, I., 1986. *N*-acetylcysteine: a drug with an interesting past and a fascinating future. Respiration 50, 26–30.